The Patent Office

EP 99/03344

INVESTOR IN PEOPLE

09/463098

EP99/3344

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP9 1RH

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 18 May 1999

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
19 MAY 1998
RECEIVED BY HAND

The Patent Office

9810756.8

20MAY98 E361824-1 D00060
P01/7700 25.00 - 9810756.8

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — SMW/FP5691266

2. Patent application number *(The Patent Office will fill in this part)* — 19 MAY 1998 — **9810756.8**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P. ANGELETTI SPA
VIA PONTINA KM. 30.600
00040 POMEZIA (ROMA)
ITALY

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — IT

T439219EC1

4. Title of the invention — MIMOTOPES OF HYPERVARIABLE REGION 1 OF THE E2 GLYCOPROTEIN OF HCV AND USES THEREOF

5. Name of your agent *(if you have one)* — MEWBURN ELLIS

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

YORK HOUSE
23 KINGSWAY
LONDON
WC2B 6HP

Patents ADP number *(if you know it)* — 109006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month /year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	81
Claim(s)	20
Abstract	1
Drawing(s)	8 +6

10. If you are also filing any of the following, state

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(Please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature Date
19 May 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

SEÁN M. WALTON 0171 240 4405

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed it it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

MIMOTOPES OF HYPERVARIABLE REGION 1 OF THE E2 GLYCOPROTEIN OF HCV AND USES THEREOF

The present invention is concerned with peptides, specifically peptides which are mimotopes of the hypervariable region 1 (HVR1) of the putative envelope protein E2 of hepatitis C virus (HCV). Employing a combination of techniques the present inventors have devised a large number of peptides with sequences based on consensus analysis of naturally occurring HVR1 sequences and experimental determination of cross-reactivity to antibodies against different isolates, none of which peptides occurs in nature. The peptides are individually useful in raising and obtaining antibodies, for *in vitro* (e.g. diagnostic) and *in vivo* purposes, and libraries of peptides are useful in identifying peptides of particular cross-reactivity with antibodies able to bind a plurality of HVR1's of different HCV strains. Peptides may be used in themselves or as part of fusion proteins, for instance in recombinant HCV E2 polypeptides, which may be incorporated into recombinant HCV particles.

The HVR1 region of HCV is the most variable antigenic fragment in the whole viral genome and is mainly responsible of the large inter and intra-individual heterogeneity of the infecting virus. It contains a principal neutralization

epitope and has been proposed as the major player in the mechanism of escape from host immune response. Since anti-HVR1 antibodies are the only species shown to possess protective activity up to date, the development of an efficient prevention therapy is a very difficult task.

In devising the present invention, the inventors approached the problem of the HVR1 variability by deriving a consensus profile from more than two hundred HVR1 sequences from different viral isolates and used this consensus as a template for generating a vast repertoire of synthetic HVR1 surrogates. These were provided as fusions to the major coat protein VIII of M13 bacteriophage for display on the surface of bacteriophage particles. This library was affinity selected using many different sera from infected patients. Phage were identified which displayed high frequency of reactivity with patients' sera, but not with sera from uninfected controls. The selected sequences were shown to bind serum antibodies cross-reacting with a large panel of peptides reproducing the HVR1 from natural HCV variants.

In these "mimotopes" was identified a sequence pattern responsible for the observed cross-reactivity. When injected in experimental animals, the mimotopes with the highest cross-reactivity induced antibodies able to recognise the same panel of natural HVR1 variants.

Hepatitis C virus (HCV) is the major etiologic agent of both blood-transfusion-associated and sporadic non-A non-B hepatitis worldwide, with an estimated prevalence between 0.4 and 2% in the blood donor population (Choo et al., 1989). HCV infection leads to viral persistence and chronic disease in at least 70% of cases, among which a significant proportion eventually develops cirrhosis and hepatocellular carcinoma (for a review see H. Alter, 1995). In spite of the availability of reliable serological tests for HCV diagnosis, community-acquired infection is still common and causes significant morbidity and mortality worldwide (Mast and Alter, 1993). In addition, interferon treatment, which is the only anti-viral therapy available at the moment, is effective only in 20-30% of patients (Fried and Hoofnagle, 1995). Thus, development of an HCV vaccine represents a high priority project in the field.

The high frequency with which the virus establishes a persistent infection, despite a wide array of humoral and cell-mediated host immune responses, raised in the past some concerns about the existence of a protective immunity against HCV (Farci et al., 1992). As a matter of fact, protective immunity against challenge with homologous virus could be induced by vaccination of chimpanzees (the only other species susceptible to HCV infection) using recombinant forms of the putative envelope proteins E1 and E2 (Choo et al., 1994).

However, it remains to be established how effective this response would be against heterologous viral inocula.

HCV exists in the bloodstream of infected patients as a quasispecies (Weiner et al., 1991; Martell et al., 1992; Martell et al., 1994; Kurosaki et al., 1994; Bukh et al., 1995) which fluctuates during the course of the disease mainly as a result of immune pressure (Weiner et al., 1992; Kato et al., 1993; Kojima et al., 1994; Shimizu et al., 1994; van Doorn et al., 1995; Weiner et al., 1995). The emerging view is that chronic infection by HCV is not due to lack of humoral or cellular responses, but rather to such responses being rendered ineffective by the high mutation rate of the virus which leads to the emergence of escape variants.

The existence of neutralising antibodies and their role in protection from viral infection was documented by *ex vivo* neutralization of a pedigreed viral inoculum prior to injection into chimpanzees (Farci et al., 1994). This notwithstanding, neutralising antibodies were isolate-specific and seemed to be able to block only viral variants which were present before the onset of the corresponding humoral response (Farci et al., 1994). Even if the specificity of such neutralising antibodies is not well defined, both immunological and molecular evidence indicate that epitopes recognised by neutralising antibodies are localised in the hypervariable region 1 (HVR1) of the HCV

genome (Farci et al., 1994). This consists of the N-terminal 27 amino acids of the E2 glycoprotein, the most variable region of the whole HCV polyprotein (Weiner et al., 1991). Direct proof for the role of anti-HVR1 antibodies in virus clearance came recently from *ex vivo* neutralization experiments. A rabbit anti-HVR1 hyperimmune serum raised against the predominant variant of an infectious HCV inoculum abolished its infectivity in one chimp, and partially protected a second animal by blocking propagation of the major variant present in the inoculum (Farci et al., 1996).

Thus, the evidence is that the HVR1 contains a principal neutralization determinant for HCV, and that it should constitute an essential component of an acellular HCV vaccine if one could surmount the problem of its variability. Relevant to this issue is the observation that anti-HVR1 antibodies from human sera display some degree of cross-reactivity to different HVR1 variants (Scarselli et al., 1995).

WO94/26306 (Chiron Corporation) discloses an attempt at identifying a consensus sequence within the HVR1 of HCV, based on sequence comparison on the 90 strains said to have been known as of 12 May 1993. The disclosed formula is of a peptide including the following sequence: aa1-aa2-aa3-aa4-aa5-aa6, wherein aa1 is S, G, A, D, K , R or T; aa2 is L, F, I, M or W; aa3 is F or L; aa4 is any amino acid; aa5 is any amino acid;

and aa6 is G or A; with the proviso that the motif is not contained within a 31 amino acid sequence of a naturally occurring E2HV domain of an HCV isolate known as of May 12, 1993. In a further embodiment, aa7 is present and attached to aa6; aa7 being A, P or S. The 6 amino acid motif represents around 55,000 different sequence. The 7 amino acid motif represents around 165,000 different sequences.

Aspects of the present invention are based in part on a close inspection of the variability in HVR1 revealing that some positions of the HVR1 are less variable than others, suggesting that the actual structural, and immunological variability is more limited than that suggested by the heterogeneity in primary sequence. The invention is concerned in various aspects with providing "synthetic variants" of the HCV HVR1 which are immunologically similar to a plurality, preferably a great number of natural HVR1 variants and, therefore, may be used to induce neutralising antibodies which cross-react with different HCV variants, preferably most or all. As explained further below, the formulae arrived at for peptides of the present invention differs from that provided in WO94/26306, and is based on actual cross-reactivity scoring rather than just sequence comparison.

Phage displayed peptide libraries offer the unique chance to rapidly survey large collections of peptidic sequences (10^8 or more) through a cyclic selection/rescue/amplification procedure. They allow identification of ligands for any type of ligate ranging from linear peptides to folded protein domains, and even carbohydrates (Cortese et al., 1994, Cortese et al., 1996). These ligands are true mimotopes as they do not necessarily share the same amino acid sequence of the original epitope, but they mimic its binding properties. A strategy for the identification of disease-specific phage-displayed mimotopes was reported previously, which avails itself only of clinically characterized sera from immune and non immune individuals (Folgori et al., 1994, hereby incorporated by reference). Furthermore, disease-specific mimotopes proved to be good immunogenic mimics of the natural antigen as they were able to induce a specific immune response to the natural antigen when injected into different animals (Folgori et al., 1994, and Meola et al., 1995, (both hereby incorporated by reference) Prezzi et al., 1996, Mecchia et al., 1996). Thus, phage libraries may be used as a source of artificial ligands recognised by disease-specific antibodies, with the advantage that additional desirable features can be built-in, providing that they can be selected for during library enrichment.

In making the present invention, the inventors approached the problem of the HVR1 variability by generating a vast repertoire of HVR1 surrogates as fusion to the major coat protein (pVIII) of bacteriophage M13. Using the power of selection and many sera from clinically characterized HCV infected individuals peptides were isolated which revealed to be good antigenic and immunogenic mimics of a large number of naturally occurring HCV variants.

Experimental details are provided below.

In accordance with various aspects of the present invention there are provided libraries of peptides containing large numbers of different peptides, individual peptides which contain epitopes cross-reactive with a plurality of HCV HVR1 epitopes, and mixtures of different such peptides.

One aspect of the present invention provides a library of peptide conforming with the following consensus profile:

Q	T	H	V	T	G	G	S	A	A	R	T	T	S	G	L	T	S	L	F	S	P	G	A	S	Q	N
T		T	T	V			V	Q	G	H	A	A	H	S		V	G			R	L		P	K		K
		R					Q	V	S		Q	V	R	R			R				S		S	Q		
																					Q					

This profile represents a total of 9 x 10^7 individual sequences, i.e. a number very close to the upper practical limit (about 10^8) of current DNA cloning and transformation techniques. As described below, this consensus profile was used for the construction of a 27aa peptide library by cloning a degenerated synthetic oligonucleotide as a fusion to the 5' end of the gene coding for the major coat protein (pVIII) in a phagemid vector for M13. The library was extensively screened using human sera, and more than one hundred different clones (mimotopes) were selected for their characteristic to specifically recognise human anti HCV-HVR1 antibodies. Nearly all these mimotopes have different amino acid sequences and none of them could be found to correspond to published (up to January 98) natural HVR1.

In a preferred embodiment of a peptide library according to the present invention there are at least about 10^5 different peptides present, preferably at least about 10^6 different peptides, more preferably at least about 10^7, e.g. about 9 x 10^7 different peptides.

A library of peptides may be displayed on the surface of bacteriophage, particularly filamentous bacteriophage such as fd or M13, for instance as fusions with the major coat protein (pVIII) of such bacteriophage. Phage display of peptides is standard in the art and its power lies in the fact that

bacteriophage particles are constructed so that packaged within each particle is nucleic acid encoding the peptide displayed on its surface. Following selection of phage particles displaying a peptide of interest, such as a peptide able to bind one or more antibodies (e.g. antibodies able to bind a number of epitopes of HVR1 of different strains of HCV), the nucleic acid encoding the displayed peptide can be retrieved and used in production of further peptide with that amino acid sequence.

In the experimental work described below, the inventors tested mimotopes in a library according to the present invention with a panel of human sera, and individual mimotopes were characterised as having a different overall frequency of reactivity with the sera. The 24 clones that only reacted with less than 3 sera were defined as "weak" while the 27 reacting with more than 11 sera were defined as "strong".

Statistical analysis of the consensus sequences of "strong" and "weak" clones, lead to the discovery of a sequence motif in the HVR1 that is correlated with high frequency of reaction with human sera, crossreactivity with human anti HVR1 antibodies and induction of highly crossreactive sera in experimental animals.

Peptides according to the present invention, and mixtures

thereof, may be defined as follows, further explanation of which is given below in the experimental section:

(1) - A library of peptides fully described by the following formula ("Formula I"):

Q	T	H	V	T	G	G	S	A	A	R	T	T	S	G	L	T	S	L	F	S	P	G	A	S	Q	N
T		T	T	V			V	Q	G	H	A	A	H	S		V	G			R	L		P	K		K
		R					Q	V	S		Q	V	R	R			R				S		S	Q		
																					Q					

which may be written as

(aa1)T(aa3)(aa4)(aa5)GG(aa8)(aa9)(aa10)(aa11)(aa12)(aa13)(aa14)(aa15)L(aa17)(aa18)LF(aa21)(aa22)G(aa24)(aa25)Q(aa27)

wherein aa1 is Q or T; aa3 is H, T or R; aa4 is V or T; aa5 is T or V; aa8 is S, V or Q;aa9 is A, Q or V; aa10 is A, G or S; aa11 is R or H; aa12 is T, A or Q; aa13 is T, A or V; aa14 is S, H or R; aa15 is G, S or R; aa17 is T or V; aa18 is S, G or R; aa21 is S or R; aa22 is P, L, S or Q; aa24 is A, P or S; aa25 is S, K or Q; aa27 is N or K.

(2) - 27 "strong" peptides obtainable from such a library are preferred peptides according to various aspects of the present invention, having an amino acid sequence as follows:

2.11	QT	H	TVGGVQG	R	QAHSLT	S	LF	S	P	G	A	SQN	*
D6	QT	T	TTGGQVS	H	ATHGLT	G	LF	S	L	G	P	QQK	*
D18	QT	H	TTGGSAS	H	QASGLT	R	LF	S	Q	G	P	SQN	*
F63	QT	H	VVGGQQG	R	QVSSLV	S	LF	S	P	G	A	SQK	*
G31	TT	H	TVGGSVA	R	QVHSLT	G	LF	S	P	G	P	QQK	*
L13	QT	H	TVGGSQA	H	AAHSLT	R	LF	S	P	G	S	SQN	*
M69	QT	T	VVGGSQA	R	AAHGLV	S	LF	S	L	G	S	KQN	*
Z61	QT	H	VVGGVQG	R	QTSGLV	G	LF	S	P	G	S	KQN	*
R9	QT	T	VVGGSQS	H	TVRGLT	S	LF	S	P	G	A	SQN	
B26	TT	T	TTGGQAG	H	QAHSLT	S	LF	S	P	G	A	SQK	
B22	QT	H	VVGGVQS	H	QTSGLT	S	LF	S	P	G	A	SQK	
B35	QT	H	TTGGVQG	H	QTSRLT	S	LF	S	P	G	P	SQN	
D29	TT	T	VVGGQAA	H	QTHSLT	S	LF	S	P	G	A	KQN	
D33	TT	T	TTGGQQS	H	TVHGLV	G	LF	S	P	G	S	KQN	
E26	QT	H	TVGGVQA	H	TVRGLT	S	LF	S	P	G	S	SQN	
F80	QT	H	TTGGQAG	H	TASSLT	G	LF	S	P	G	A	KQN	
F19	QT	T	TVGGVAS	H	QAHSLT	G	LF	S	P	G	A	KQK	
F78	QT	H	TTGGQAG	H	QAHSLT	G	LF	S	P	G	A	KQN	
H1	QT	H	TTGGVVG	H	ATSGLT	S	LF	S	P	G	P	SQK	
L76	TT	T	TVGGQAS	H	QTSSLT	G	LF	S	P	G	S	KQN	
M27	QT	T	TTGGVAS	H	AAHRLT	S	LF	S	P	G	P	QQK	
M122	QT	T	TTGGSAS	H	AVSSLT	G	LF	S	P	G	S	KQN	
M129	QT	T	VVGGSAG	H	TASSLV	G	LF	S	P	G	S	KQN	
M119	TT	T	TVGGQAS	H	TTSSLT	G	LF	S	P	G	S	QQN	

R5 QT H TTGGQAS H QVSSLV S LF S P G A KQK

R6 TT T TTGGQVG H QTSGLT G LF S P G A QQN

R27 TT H VVGGSAS H AVRGLT S LF S P G S SQN

Further preferred peptides according to the present invention have any of the following sequences:

B14 QT T VTG_QAS H TTSSLT G LF S P G A SQK

B33 aT H aTGGQAA H STHSLT S LF S P G A SQK

F81 QT H VTGGSAA H QTgGLT G LF S P G P KQN

B18 QT T VVGGQAS H _VSRLT G LF S P G S SQK

L72 QT T T____AA H TTSGLT G LF S P G A KQN

D20 QT H VTG_VAG R QTSGLV S LF S P G S SQN

D30 Q_ _ __GGVQG H TTSSLV G LF S P G S QQN

The lower case letters are used to identify amino acid residues that vary from Formula 1, while the underlined spaces are included to signify deletions compared with Formula 1, though the flanking amino acids are of course contiguous in the relevant peptides.

These are variants of peptides obtainable from a library in accordance with the present invention, not themselves conforming with Formula I. They were identified in the course

of the experiments identified below and originated by PCR errors during library amplification. (See Materials and Methods, "Construction of the HVR1 library".)

(3) - A "strong consensus" ("Formula II"), derived from the consensus of the highly cross-reactive peptides of (2) above.

The statistical analysis of the frequencies of aa in any position in the 27 "strong" in comparison with the frequency in 25 "weak" is shown in Table II, and discussed further below in the experimental section.

Formula II:

QT(aa3)TVGGQQS(aa11)QVHSLT(aa18)LF(aa21)(aa22)G(aa24)SQN

where: aa3 is H or T; aa11 is H or *R*; aa18 is G, S or *R*; aa21 is S; aa22 is P, *L* or *Q*; aa24 is A, S or P;

which may also be written:

QT **H** TVGGQAS **H** QASSLT **S** LF **S** **P** G **A** KQN

T *R* G *L* **S**

R *Q* **P**

Residues in italics are included because although they have low frequencies they are found in some of the best reactive

mimotopes tested (highlighted with an asterisk among the 27 "strong" peptides at II above.

The 27 mimotopes used to derive Formula II are not in it.

108 peptides conform to Formula II and each is an aspect of the invention. The sequences are:

1 QTHTV GGQAS HQASS LTSLF SPGAK QN
2 QTHTV GGQAS HQASS LTSLF SPGSK QN
3 QTHTV GGQAS HQASS LTSLF SPGPK QN
4 QTHTV GGQAS HQASS LTSLF SLGAK QN
5 QTHTV GGQAS HQASS LTSLF SLGSK QN
6 QTHTV GGQAS HQASS LTSLF SLGPK QN
7 QTHTV GGQAS HQASS LTSLF SQGAK QN
8 QTHTV GGQAS HQASS LTSLF SQGSK QN
9 QTHTV GGQAS HQASS LTSLF SQGPK QN
10 QTHTV GGQAS HQASS LTGLF SPGAK QN
11 QTHTV GGQAS HQASS LTGLF SPGSK QN
12 QTHTV GGQAS HQASS LTGLF SPGPK QN
13 QTHTV GGQAS HQASS LTGLF SLGAK QN
14 QTHTV GGQAS HQASS LTGLF SLGSK QN
15 QTHTV GGQAS HQASS LTGLF SLGPK QN
16 QTHTV GGQAS HQASS LTGLF SQGAK QN
17 QTHTV GGQAS HQASS LTGLF SQGSK QN

18 QTHTV GGQAS HQASS LTGLF SQGPK QN

19 QTHTV GGQAS HQASS LTRLF SPGAK QN

20 QTHTV GGQAS HQASS LTRLF SPGSK QN

21 QTHTV GGQAS HQASS LTRLF SPGPK QN

22 QTHTV GGQAS HQASS LTRLF SLGAK QN

23 QTHTV GGQAS HQASS LTRLF SLGSK QN

24 QTHTV GGQAS HQASS LTRLF SLGPK QN

25 QTHTV GGQAS HQASS LTRLF SQGAK QN

26 QTHTV GGQAS HQASS LTRLF SQGSK QN

27 QTHTV GGQAS HQASS LTRLF SQGPK QN

28 QTHTV GGQAS RQASS LTSLF SPGAK QN

29 QTHTV GGQAS RQASS LTSLF SPGSK QN

30 QTHTV GGQAS RQASS LTSLF SPGPK QN

31 QTHTV GGQAS RQASS LTSLF SLGAK QN

32 QTHTV GGQAS RQASS LTSLF SLGSK QN

33 QTHTV GGQAS RQASS LTSLF SLGPK QN

34 QTHTV GGQAS RQASS LTSLF SQGAK QN

35 QTHTV GGQAS RQASS LTSLF SQGSK QN

36 QTHTV GGQAS RQASS LTSLF SQGPK QN

37 QTHTV GGQAS RQASS LTGLF SPGAK QN

38 QTHTV GGQAS RQASS LTGLF SPGSK QN

39 QTHTV GGQAS RQASS LTGLF SPGPK QN

40 QTHTV GGQAS RQASS LTGLF SLGAK QN

41 QTHTV GGQAS RQASS LTGLF SLGSK QN

42 QTHTV GGQAS RQASS LTGLF SLGPK QN

43 QTHTV GGQAS RQASS LTGLF SQGAK QN

44 QTHTV GGQAS RQASS LTGLF SQGSK QN

45 QTHTV GGQAS RQASS LTGLF SQGPK QN

46 QTHTV GGQAS RQASS LTRLF SPGAK QN

47 QTHTV GGQAS RQASS LTRLF SPGSK QN

48 QTHTV GGQAS RQASS LTRLF SPGPK QN

49 QTHTV GGQAS RQASS LTRLF SLGAK QN

50 QTHTV GGQAS RQASS LTRLF SLGSK QN

51 QTHTV GGQAS RQASS LTRLF SLGPK QN

52 QTHTV GGQAS RQASS LTRLF SQGAK QN

53 QTHTV GGQAS RQASS LTRLF SQGSK QN

54 QTHTV GGQAS RQASS LTRLF SQGPK QN

55 QTTTV GGQAS HQASS LTSLF SPGAK QN

56 QTTTV GGQAS HQASS LTSLF SPGSK QN

57 QTTTV GGQAS HQASS LTSLF SPGPK QN

58 QTTTV GGQAS HQASS LTSLF SLGAK QN

59 QTTTV GGQAS HQASS LTSLF SLGSK QN

60 QTTTV GGQAS HQASS LTSLF SLGPK QN

61 QTTTV GGQAS HQASS LTSLF SQGAK QN

62 QTTTV GGQAS HQASS LTSLF SQGSK QN

63 QTTTV GGQAS HQASS LTSLF SQGPK QN

64 QTTTV GGQAS HQASS LTGLF SPGAK QN

65 QTTTV GGQAS HQASS LTGLF SPGSK QN

66 QTTTV GGQAS HQASS LTGLF SPGPK QN

67 QTTTV GGQAS HQASS LTGLF SLGAK QN

68 QTTTV GGQAS HQASS LTGLF SLGSK QN

69 QTTTV GGQAS HQASS LTGLF SLGPK QN

70 QTTTV GGQAS HQASS LTGLF SQGAK QN

71 QTTTV GGQAS HQASS LTGLF SQGSK QN

72 QTTTV GGQAS HQASS LTGLF SQGPK QN

73 QTTTV GGQAS HQASS LTRLF SPGAK QN

74 QTTTV GGQAS HQASS LTRLF SPGSK QN

75 QTTTV GGQAS HQASS LTRLF SPGPK QN

76 QTTTV GGQAS HQASS LTRLF SLGAK QN

77 QTTTV GGQAS HQASS LTRLF SLGSK QN

78 QTTTV GGQAS HQASS LTRLF SLGPK QN

79 QTTTV GGQAS HQASS LTRLF SQGAK QN

80 QTTTV GGQAS HQASS LTRLF SQGSK QN

81 QTTTV GGQAS HQASS LTRLF SQGPK QN

82 QTTTV GGQAS RQASS LTSLF SPGAK QN

83 QTTTV GGQAS RQASS LTSLF SPGSK QN

84 QTTTV GGQAS RQASS LTSLF SPGPK QN

85 QTTTV GGQAS RQASS LTSLF SLGAK QN

86 QTTTV GGQAS RQASS LTSLF SLGSK QN

87 QTTTV GGQAS RQASS LTSLF SLGPK QN

88 QTTTV GGQAS RQASS LTSLF SQGAK QN

89 QTTTV GGQAS RQASS LTSLF SQGSK QN

90 QTTTV GGQAS RQASS LTSLF SQGPK QN

91 QTTTV GGQAS RQASS LTGLF SPGAK QN

92 QTTTV GGQAS RQASS LTGLF SPGSK QN

93 QTTTV GGQAS RQASS LTGLF SPGPK QN

94 QTTTV GGQAS RQASS LTGLF SLGAK QN

95 QTTTV GGQAS RQASS LTGLF SLGSK QN

96 QTTTV GGQAS RQASS LTGLF SLGPK QN

97 QTTTV GGQAS RQASS LTGLF SQGAK QN

98 QTTTV GGQAS RQASS LTGLF SQGSK QN

99 QTTTV GGQAS RQASS LTGLF SQGPK QN

100 QTTTV GGQAS RQASS LTRLF SPGAK QN

101 QTTTV GGQAS RQASS LTRLF SPGSK QN

102 QTTTV GGQAS RQASS LTRLF SPGPK QN

103 QTTTV GGQAS RQASS LTRLF SLGAK QN

104 QTTTV GGQAS RQASS LTRLF SLGSK QN

105 QTTTV GGQAS RQASS LTRLF SLGPK QN

106 QTTTV GGQAS RQASS LTRLF SQGAK QN

107 QTTTV GGQAS RQASS LTRLF SQGSK QN

108 QTTTV GGQAS RQASS LTRLF SQGPK QN

(4) - A further library of peptides within the library of Formula I, including the sequences of Formula II, defining 2.5 x 10^6 sequences and conforming to the following Formula III:

Q	T	**H**	T	V	G	G	Q	A	S	**H**	Q	A	S	S	L	T	**S**	L	F	**S**	**P**	G	**A**	K	Q	N
T		**T**	V	T			S	Q	G		A	T	H	G		V	**G**							**S**	S		K
							V	V	A		T	V	R	R										**P**	Q		

A peptide according to the present invention may be provided in a fusion with additional amino acids. Additional amino acids may be fused at one or both of the N-terminus and the C-terminus of the peptide. The additional amino acids may be an amino acid sequence that is not a fragment of HCV E2 protein, or may be an amino acid sequence that is part of that protein. Furthermore, a fusion including a peptide according to the present invention may include a HCV E2/NS1 protein with the peptide amino acid sequence in the HVR1 position, i.e. such that the mimotope HVR1 peptide of the invention substitutes for the natural HVR1 sequence. Another way of expressing this is to refer to a "recombinant HCV E2/NS1 protein in which a peptide of the present invention is substituted for the HVR1". As noted below, nucleic acid encoding peptides and polypeptides, including fusions, according to invention are provided as further aspects of the invention, as is a recombinant HCV genome including a nucleotide sequence encoding a peptide of the invention, for instance within the E2/NS1 coding sequence to provide for production of a recombinant HCV E2/NS1 protein in which a peptide of the invention is

substituted for the HVR1 and incorporation of the recombinant protein into an assembled HCV particle. A recombinant HCV particle including one or more peptides or polypeptides as disclosed herein is provided as a further aspect of the present invention.

Generally, a peptide according to the present invention is immunogenic or able to raise an immune response on administration to an individual or includes an epitope immunologically cross-reactive with an epitope of a plurality of strains of HCV.

Another aspect of the present invention provides a method of obtaining one or more peptides containing an epitope immunologically cross-reactive with an epitope in the HVR1 of an HCV strain, the method including bringing into contact a library of peptides as disclosed and an antibody molecule able to bind said HVR1 of an HCV strain, and selecting one or more peptides of the library able to bind said antibody molecule.

The peptide or peptides selected may contain an epitope immunologically cross-reactive with the HVR1 of a plurality of strains of HCV.

Such a method may include bringing into contact a library of peptides and a plurality of antibody molecules collectively

able to bind the HVR1 of a plurality of strains of HCV. In one embodiment, said plurality of antibody molecules is derived from sera of individuals infected with HCV.

As noted, said library may be displayed on the surface of bacteriophage particles, each particle containing nucleic acid encoding the peptide displayed on its surface. Following selection, nucleic acid may be taken from a bacteriophage particle displaying a said selected peptide. Nucleic acid with the sequence of nucleic acid taken from a bacteriophage particle displaying a said selected peptide may be used in production of such a peptide by means of expression (using recombinant DNA technology as standard in the art and discussed further below).

A peptide with the amino acid sequence of a said selected peptide may provided in isolated form, e.g. after its production by expression from encoding nucleic acid. As noted further below, one or more peptides in accordance with the present invention may be provided by peptide synthesis.

A plurality of peptides each with the amino acid sequence of a different selected peptide may provided in isolated form, individually or in a mixture.

A selected peptide or selected peptides may each have an amino acid sequence according to the Formula II given above. All 108 of the different peptides according to Formula II may

be provided as a mixture, and furthermore each individually represents an aspect of the present invention. Each peptide of these 108 has a high probability of being cross-reactive with epitopes in the HVR1 of the E2/NS2 protein of a number of strains of HCV, and therefore is particularly useful for obtaining antibodies or otherwise raising an immune response.

A composition according to the present invention may include a plurality of peptides obtainable from a mixture of the 108 peptides of Formula II. Such a composition may include from 2 to about 20, 15, 10, 9, 8, 7, 6, 5, 4 or 3 different peptides obtainable from said mixture.

Preferred peptides which may be provided in a mixture or individually include those denoted G31, F78, R9, D6, M122 and H1 of which the amino acid sequences are shown in Figure 7(A) (R9 has the same sequence as B16).

Preferred mixtures included peptides R9, F78, H1 and D6 ("MIX1"), include peptides M122 and G31 ("MIX2"), or include peptides G31, F78, R9, D6, M122 and H1 ("MIX3").

Immunological cross-reactivity of each peptide of the invention with the HVR1 of HCV strains can be assessed experimentally, as exemplified below. Various mixtures of these peptides may also be made and similarly tested, again as experimentally exemplified below.

Linear or branched (e.g. MAP) peptides and polypeptides (e.g. fusion molecules including a peptide as discussed) in accordance with the present invention may be made using any of a variety of techniques at the disposal of the ordinary person skilled in the art.

Linear or branched peptides may be synthesized using standard peptide chemistry such as by the common method employing Fmoc (Fluorenilmetil-ossicarbonil)t-Bu (*tert*-butil), as described in Atherton and Sheppard (1989), *Solid Phase Peptide Synthesis, a Practical Approach*, IRL Press, Oxford.

A convenient way of producing a peptide or polypeptide according to the present invention is to express nucleic acid encoding it, by use of the nucleic acid in an expression system.

Accordingly, the present invention also encompasses a method of making a peptide or polypeptide (as disclosed), the method including expression from nucleic acid encoding the peptide or polypeptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the polypeptide. Peptides and polypeptides may also be expressed in *in vitro* systems, such as reticulocyte lysate.

Polynucleotides encoding peptides and polypeptides according to the present invention represent further aspects of the invention.

In one aspect there is provided a polynucleotide encoding a peptide as disclosed. In a further aspect, there is provided a polynucleotide encoding a fusion as disclosed, particularly a HCV E2/NS1 protein including the amino acid sequence of a peptide of the invention in the HVR1 position. In a further aspect, there is provided a recombinant HCV genome including a nucleotide sequence encoding a peptide according to the invention or a fusion as disclosed, particularly a HCV E2/NS1 protein with the relevant peptide amino acid sequence in the HVR1 position.

In a still further aspect, a polynucleotide is provided which includes a plurality of nucleotide sequences encoding peptides or polypeptides according to the invention. This allows for production of a mixture of peptides or polypeptides in a single expression reaction.

Nucleic acid encoding a peptide or polypeptide according to the present invention may be used in nucleic acid immunisation in order to raise an immune response in a mammal, such as a human individual for a therapeutic or prophylactic purpose, or a non-human mammal for such a purpose or in order

to produce antibodies for subsequent manipulation and/or use (e.g. in diagnostic or therapeutic contexts as discussed further below.)

Nucleic acid encoding a peptide or polypeptide according to the present invention may be used in a method of gene therapy, in prevention and/or treatment of HCV infection. This requires use of suitable regulatory elements for expression and a suitable vector for deliver of the expression unit (coding sequence and regulatory elements) to host cells. A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see e.g. US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses. A variety of adenovirus and adeno-associated viral vectors have been developed. Alternatives to viral vectors include transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

Host cells containing nucleic acid encoding a peptide or polypeptide (or mixture thereof) according to the present invention may themselves be used in therapeutic or prophylactic treatment of individuals for or against HCV infection (i.e. therapeutic treatment of an individual with an HCV infection or

prophylactic treatment of an individual prior to HCV infection).

Nucleic acid is generally provided as DNA or RNA, though may include one or more nucleotide analogues, and may be wholly or partially synthetic. Nucleic acid molecules and vectors according to the present invention may be provided in isolated and/or purified form, e.g. in substantially pure or homogeneous form. The term "isolate" may be used to reflect all these possibilities. Where a DNA sequence is specified, e.g. with reference to a figure, unless context requires otherwise the RNA equivalent, with U substituted for T where it occurs, is encompassed.

Where it is desired to express a peptide or polypeptide from encoding nucleic acid, the nucleic acid includes appropriate regulatory control sequences. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of

nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons, 1992.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others. A common, preferred bacterial host is *E. coli*.

A further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. The nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell.

A still further aspect provides a method which includes introducing the nucleic acid into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as

"transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed. Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded peptide or polypeptide is produced. If the peptide or polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a peptide or polypeptide may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a

composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see below).

A peptide or polypeptide according to the present invention may be used as an immunogen or otherwise in obtaining binding antibodies. Antibodies are useful in purification and other manipulation of polypeptides and peptides, diagnostic screening and therapeutic contexts, including passive immunisation. This is discussed further below.

According to a further aspect of the present invention there is provided a method of obtaining one or more antibody molecules containing a binding site able to bind an epitope in the HVR1 of a plurality of HCV strains, the method including bringing into contact a population of antibody molecules and a peptide according to the present invention, and selecting one or more antibody molecules of the population able to bind said peptide.

The method may involve bringing the population of antibodies into contact with a plurality of peptides according to the invention.

As noted, the peptides may be provided in a fusion with

additional amino acids.

The peptide or peptides may be administered to a non-human mammal to bring them into contact with a population of antibody molecules produced by the mammal's immune system, then one or more antibody molecules able to bind the peptide or peptides may be taken from the mammal, or cells producing such antibody molecules may be taken from the mammal.

The mammal may be sacrificed.

If cells are taken from the mammal, antibody molecules may be taken from said cells or descendants thereof. Such descendants in particular may include hybridoma cells.

Instead or as well as immunising an animal, a method of obtaining antibodies as disclosed may involve displaying the population of antibody molecules on the surface of bacteriophage particles, each particle containing nucleic acid encoding the antibody molecule displayed on its surface. Nucleic acid may be taken from a bacteriophage particle displaying an antibody molecule able to bind a peptide or peptides of interest, for manipulation and/or use in production of the encoded antibody molecule or a derivative thereof (e.g. a fusion protein, a molecule including a constant region or other amino acids, and so on). Instead of using bacteriophage for display, ribosomes or polysomes may be used, e.g. as disclosed in US-A-5643768, US-A-5658754, WO95/11922.

Antibody molecules may be provided in isolated form, either individually or in a mixture. A plurality of antibody molecules may be provided in isolated form.

Preferred antibodies according to the invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention. Indeed, polyclonal mixtures able to bind one or more peptides or polypeptides according to the present invention are preferred in some embodiments, as discussed. Thus, the present invention in a further aspect is directed to a mixture of different antibodies able to bind one or more peptides or polypeptides according to the invention. Such a mixture may be provided in a composition including at least one additional component, such as a pharmaceutically acceptable excipient or vehicle.

The present invention also extends to methods of obtaining and/or raising antibodies to one or more peptides or polypeptides of the invention. Such methods may include administering a peptide or polypeptide or mixture of peptides or polypeptides to a mammal in order to raise an antibody response. In a therapeutic or prophylactic context the mammal may be human or non-human. For the production of antibodies or

antibody-producing cells to be isolated and used for any of a variety of purposes, a step of sacrificing a non-human mammal may be included. Such a non-human mammal may be for example mouse, rat, rabbit, dog, cat, pig, horse, donkey, goat, sheep, camel, Old World monkey, chimpanzee or other primate. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to peptide or polypeptide of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, Nature, 357:80-82, 1992).

The production of polyclonal and monoclonal antibodies is well established in the art. Monoclonal antibodies can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB-A-2188638 or EP-A-239400. Humanised antibodies in which CDRs from a non-human source are grafted onto human framework regions, typically with the alteration of some of the framework amino acid residues, to provide

antibodies which are less immunogenic than the parent non-human antibodies, are also included within the present invention. A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes , which may or may not alter the binding specificity of antibodies produced. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using bacteriophage which display functional immunoglobulin binding domains on their surfaces - for instance see WO92/01047 - or ribosomes/polysomes as noted above. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic

molecules and molecules whose shape mimics that of an antibody enabling it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')2 fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample (e.g. in a diagnostic test) may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility.

The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or

biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

Antibodies according to the present invention may be used in screening for the presence of a peptide or polypeptide, for example in a test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a peptide or polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor.

Antibodies are also useful in prophylaxis, by way of passive immunisation, and in therapy. Where antibodies are to be administered, it may be preferable to include a mixture of antibodies, such as antibodies collectively cross-reactive with a plurality of peptides according to the present invention.

Antibodies which bind a peptide in accordance with the present invention may themselves be used as immunogens in the production of anti-idiotypic antibodies. These may be used to mimic a peptide epitope in raising an immune response in an individual, e.g. for therapeutic and/or prophylactic purposes.

An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling molecules, buffer solutions, elutants and so on. Reagents may be provided within containers which protect them from the external environment, such as a sealed vial.

Diagnostic methods make use of biological samples from individuals that may contain one or more HCV strains. Examples of biological samples include fluid such as blood, plasma, serum, urine and saliva, and tissue samples.

There are various methods for determining the presence or absence in a test sample of a particular peptide or polypeptide, including methods wherein the polypeptide to be detected is an antibody.

A sample may be tested for the presence of a specific binding member such as an antibody (or mixture of antibodies) directed to one or more peptides of the invention.

Peptides according to the present invention may be used to determine the presence or absence of antibodies against HCV strains in test samples, by assessment of binding the peptides to anti-HCV E2HVR1 antibodies if present in the sample.

In theory it may be possible to identify the presence in a

sample of a binding partner for a specific binding member such as an antibody (or mixture of antibodies) directed to one or more peptides of the invention. However, to date no-one has succeeded in isolating HCV virions from a human sample. In the future, should it prove possible to identify HCV virions in human samples and/or detect such virions immunologically, peptides of the invention and particularly antibodies directed thereto will be useful in such detection.

For detection of antibodies to HCV, a biological or other sample may be tested by being contacted with one or more peptides of the invention under appropriate conditions for specific binding, before binding is determined, for instance using a reporter system as discussed. Where a panel of peptides is used, different reporting labels may be employed for each peptide so that binding of each can be determined.

A specific binding member such as a peptide may be used to isolate and/or purify its binding partner antibody from a test sample, to allow for sequence and/or biochemical analysis of the antibody. Amino acid sequencing is routine in the art using automated sequencing machines.

A typical immunoassay may involve incubating a test sample with peptides according to the invention under conditions to allow formation of immune complexes if an appropriate antibody

is present in the sample, and detecting the presence or absence of immune complex.

As noted, although not technically feasible at the moment, in principle antibodies according to the present invention may be used to determine the presence or absence of HCV strains in test samples, by assessment of binding of the antibodies to E2HVR1 epitopes if present in the sample.

A typical immunoassay may involve incubating a test sample with peptides or anti-idiotypic antibodies according to the invention under conditions to allow formation of immune complexes if an appropriate antibody is present in the sample, and detecting the presence or absence of immune complex.

A sample may be tested for the presence of an antibody directed to one or more peptides of the invention, using one or more such peptides (or polypeptide including such peptide) or one or more anti-idiotypic antibodies.

A biological or other sample may be tested by being contacted with a peptide or polypeptide or anti-idiotypic antibody under appropriate conditions for specific binding, before binding is determined, for instance using a reporter system as discussed.

The detection of formation of a binding complex in an

immunoassay in accordance with the present invention may be performed using any available technique without limitation to the scope of the invention. Some suitable techniques are described above with reference to antibody labelling. Assays may involve immobilising antibody or peptide, as the case may be, on a suitable solid phase or support, such as latex particles, magnetic or non-magnetic beads, a membrane, chip, plastic, metal, silicon or glass surface, or any other suitable material at the disposal of the skilled person. Detection may be qualitative or quantitative. One or more appropriate controls may be included, in accordance with standard practice.

As noted already, peptides, polypeptides, antibodies and nucleic acid in accordance with the present invention may be formulated into compositions, and are useful in pharmaceutical contexts. These compositions may include, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Branched peptides, such as MAP (Tam, J.P, 1988) may be used for the preparation of immunogens, either alone or linked to an appropriate carrier.

A linear peptide for use in raising an immune response may also be linked to an appropriate carrier. Various methods of coupling peptides to other molecules are known in the art,

including disulphide forming reagents (where the peptide includes a cysteine - or a cysteine is added to the peptide for this purpose), thio-ether forming coupling agents and so on. Carriers include human serum albumin (HSA), tetanus toxoid, other rather large proteins that have reasonable half-lives under physiological conditions, and stable non-proteinaceous molecules such as polysaccharides and copolymers of amino acids.

An adjuvant may be included, such as alum, oil-in-water emulsions or Freund's Adjuvant (Complete or Incomplete). Cytokines may be used to potentiate immunogenicity of the peptide or polypeptide composition.

Mimotope sequences may be cloned into the context of the HCV envelope (E2) protein in order to use the natural folding environment for correct presentation of the epitope or epitopes to the immune system.

Naked DNA may be used for immunization (see e.g. Cohen, J, 1993), and one or more mimotope sequences may be cloned into suitable vectors (see e.g. Major *et al.*, 1995). Naked DNA may be delivered using direct injection or by use of gene-guns (Yang *et al.*, 1990) or any other suitable technique.

Whether it is a polypeptide, antibody, peptide, nucleic acid molecule, small molecule or other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration may be in an immunogenic amount, that is sufficient to raise an immune (particularly antibody) response in the individual, or in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy). A prophylactic effect is sufficient to potentiate the immune response of an individual to a subsequent challenge with HCV, E2HV polypeptide, or HVR1 peptide, or to a subsequent infection with HCV, preferably in the latter case (HCV infection) to sufficient to antagonise the infection, wholly or partially. Most preferably the effect is sufficient to prevent the individual from suffering one or more clinical symptoms as a result of subsequent HCV infection, and/or protect the individual from hepatitis C. A therapeutic effect is sufficient to potentiate the immune response of an individual to pre-existing HCV infection, preferably sufficient to antagonise the infection, wholly or partially. Most preferably the effect is sufficient to ameliorate one or more clinical symptoms, and/or cure the hepatitis C and/or reduce viral titre in the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature

and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

Further aspects of the invention provide methods of treatment including administration of a peptide, mixture of peptides, antibody molecule or mixture of antibody molecules, as provided, pharmaceutical compositions including such a peptide, mixture of peptides, antibody molecule or mixture of antibody molecules, and use of such a peptide, mixture of peptides, antibody molecule or mixture of antibody molecules, in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition including formulating the specific binding member with a pharmaceutically acceptable excipient.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated and the availability

of alternative or additional treatments.

One aspect of the present invention provides use of a peptide as disclosed in the manufacture of a medicament for raising in a mammal antibodies able to bind HCV HVR1 epitopes.

Another aspect provides a method of immunising a mammal against HCV infection, the method including administering a peptide or mixture of peptides to the mammal.

A still further aspect provides a method of (passively) immunising a mammal against HCV infection, the method including administering an antibody according to the invention to the mammal, or a mixture of antibodies.

Similarly, further aspects of the invention provide a method of treating a mammal with an HCV infection, the method including administering a peptide according to the invention, or a mixture of peptides, or an antibody, or a mixture of antibodies, to the mammal.

The antibodies may be anti-idiotypic antibodies.

Aspects and embodiments of the present invention will now be illustrated further and experimentally exemplified with reference to various figures. Further aspects and embodiments of the present invention will be apparent to those of ordinary skill in the art.

In the figures:

Figure 1(A) illustrates derivation of the consensus pattern of the 234 natural variants of the HCV HVR1 sequences used in this work. Non shaded residues within the box account alone for about 80% of the observed variability. Residues are listed in decreasing order of observed frequency from top to bottom.

Figure 1(B) shows the composition in the initial HVR1 peptide library which was displayed on bacteriophage.

Figure 2 shows reactivity of phage pools yielded by the first round of affinity selection to antibodies present in the selecting sera. For each serum sample antibody recognition of the phage pools (σ1, σ4R, σ3, σ2P, σ2R, σ3R and σN), wild type phage (wt) and the unselected library (HVR1 lib) was measured. Average values (A_{405nm}) from two independent experiments have been determined.

Figure 3 shows distribution of HCV-specific phage selected from the HVR1 library as function of their frequency of reactivity with sera from infected patients. Binding is shown for phage enriched by one (top panel) or two (bottom panel) cycles of affinity selection to antibodies present in twenty human sera different from those used for the selections. For each serum, average values (A_{405nm}) from two independent experiments have been determined on the selected phage and on

wild type phage. Values were considered statistically significant when differing more than $3\sigma_{max}$ ($p<0.003$) from the background signal observed for the wild type phage. Each histogram represents the number of phage (shown on the vertical axis) reacting with the indicated number of sera expressed as percentage over total number of tested samples (horizontal axis).

Figure 4 shows that the selected mimotopes are frequently recognized by antibodies present in human sera from HCV infected patients. Binding of the selected mimotopes to antibodies present in human sera was detected by ELISA on immobilised phage. Mimotopes' names are indicated at the top of each column. For each serum (indicated on the left of each row), average values (A_{405nm}) from two independent experiments have been determined. Results are expressed as the difference between the average value of the tested phagotope and that of wild type phage. Positive values are indicated in bold. Values were considered statistically significant when differing more than $3\sigma_{max}$ ($p<0.003$) from the background signal observed for the wild type phage. The frequency of reactivity of each mimotope and that resulting from the sum of the reactivities observed with all four mimotopes are shown at the bottom of each panel.

Figure 4(A) shows reactivity of selected mimotopes with

the panel of twenty HCV patients' sera used for the screening step.

Figure 4(B) shows reactivity of selected mimotopes with an additional panel of sera from HCV-infected viremic patients.

Figure 4(C) shows reactivity with sera from non viremic patients that were scored positive for anti-HCV antibodies using commercially available kits.

Figure 5 shows correlation between the S-score and the frequency of reactivity of the selected mimotopes. The straight line represents the linear least square fit of the data. The correlation coefficient is 0.79.

Figure 6 shows that the selected mimotopes are antigenic mimics of a large number of naturally occurring HVR1. Antibodies from a pool of sera from HCV infected patients were immunopurified on MAPs reproducing the sequence of selected mimotopes (indicated at the top of the figure). Reactivity of equal amounts of the immunopurified antibodies was measured by ELISA on a representative panel of HVR1 sequences synthesized as MAPs (indicated in the left column). Average values from two independent experiments were determined. Values were considered statistically significant when two criteria were contemporarily fulfilled: (1) values were differing more than $3\sigma_{max}$ ($p<0.003$) from the background signal observed on two unrelated peptides; (2) values were differing more than $3\sigma_{max}$

($p<0.003$) from the average signal observed using ten sera from non infected individuals on each peptide representing a natural HVR1. Grey boxes indicate signals differing from those observed on the unrelated MAPs between 0.15 and 0.5 OD (405nm); black boxes indicate values differing more than 0.5 OD (405nm). The level of cross-reactivity of each pool of immunopurified antibodies is indicated at the bottom of each column.

Figure 7 shows correlation between mimotope sequence and cross-reactivity.

Figure 7(A) shows the sequences of the mimotopes used in the analysis.

Figure 7(B) shows correlation between the S-score of the mimotopes and the cross-reactivity of immunopurified human antibodies with a panel of 43 natural HVR1 sequences. The straight line represents the linear least square fit of the data. The correlation coefficient is 0.86.

Figure 8 shows that the selected mimotopes are immunogenic mimics of a large number of naturally occurring HVR1. Reactivity of sera from mice immunised with single HVR1 mimotopes (Figure 8(A)) and mixtures of mimotopes (Figure 8(B)) in the form of MAP was assayed by ELISA on the panel of natural HVR1 sequences (indicated in the left column). Immunizing mimotopes are shown in the first row. MIX1 includes mimotopes R9, F78, H1 and D6; MIX2 contains M122 and G31 peptides; MIX3

is composed of all six MAPs. Titres (defined as the dilution required to obtain half maximal signal in ELISA on the homologous peptide) are shown in the second row. Sera were diluted 1:100. Average values from two independent experiments have been determined. Values were considered statistically significant when differing more than $3\sigma_{max}$ ($p<0.003$) from the background signal observed on two unrelated peptides. Grey boxes indicate signals differing from those observed on the unrelated MAPs between 0.15 and 0.5 OD (405nm); black boxes indicate values differing more than 0.5 OD (405nm). The level of cross-reactivity of each serum is indicated at the bottom of each column.

EXAMPLE 1 - Design and construction of a specialised phage library mimicking the HVR1 variability

A multiple sequence alignment of 234 unique HVR1 sequences extracted from the sequence databases was made to characterise the variation in residue composition at each of the N-terminal 27 positions of the HCV E2 glycoprotein. A sequence pattern emerged from this analysis (Figure 1A) allowing the definition of a degenerate consensus sequence. A synthetic repertoire of HVR1 sequences was designed to contain such conserved constraints while reproducing the observed natural variability in the remaining positions.

A "consensus-profile" accounting for approximately 80% of the total sequence variability was derived by selecting the most frequent residues at each position. When similar amino acids were present at a given position, only one was chosen as representative of the variability, preferring those residues which could more effectively form interactions. For example, in position 5 both Ser and Thr are present in the natural repertoire, but only Thr was selected to design the library (Figure 1). In some cases, a residue not present in the consensus was included in the library to better mirror the overall variability. For example, Thr was included in position 3 to account for the presence of Ser, Thr, Asn in the natural repertoire of HVR1s.

The resulting final consensus profile (Figure 1B) has a complexity of 9×10^7 very close to the upper practical limit (about 10^8) of current DNA cloning and transformation techniques. The amino acid most frequently observed in the natural repertoire was always included with the exception of position 1, (where Gln and Thr were selected although Glu is the most frequently observed amino acid). Eight positions (2, 6, 7, 16, 19, 20, 23 and 26) where kept constant given the high local sequence conservation throughout the 234 natural HVR1 variants. Noteworthy also is the total absence of negatively charged residues. With the exception of position 1, where Gln

was chosen to represent the His, Glu, Asp, Gln, Asn group, no acidic residues were present within the 80% fraction. Qualitatively, the profile can be described as a generally more variable central region flanked by N-terminal and C-terminal tails containing conserved elements.

Construction of the library proceeded by cloning a degenerated synthetic oligonucleotide as a fusion to the 5' end of the gene coding for the major coat protein (pVIII) in a phagemid vector for M13 display (see Materials and Methods). About 2×10^8 independent transformants were obtained. To verify the quality and complexity of the library (HVR1 library), the inserts of fifty-six randomly chosen individual clones were sequenced. This analysis led to the following results:

(1) all clones displayed different sequences;

(2) 63% of the clones contained full-length inserts while the remaining ones had small deletions;

(3) none of the sequenced clones encoded for peptides corresponding to known HVR1 from viral isolates, searched on 15 March 1998.

From these data it was inferred that the library has a complexity close to the number of individual transformants.

EXAMPLE 2 - Identification of HVR1 mimotopes frequently

reacting with HCV patients' sera

The more complex and diverse the repertoire of antibodies used for the selection, the higher should be the probability to enrich phage recognised by many different antibodies against HVR1 epitopes. Sera from chronically infected, viremic patients appear to meet these requirements as these individuals have a rather long history of viral persistence, during which a large number of HCV variants have been generated and have challenged the immune system, presumably leading to the accumulation of a highly heterogeneous population of anti-HVR1 antibodies.

Eight sera from chronic patients infected by viruses of five different genotypes: 1a, 1b, 2a, 2b, 3a (Simmonds et al., 1993) were used to perform six affinity selections of the HVR1 library (Table 1). As control, a serum from a non infected individual was also used. Pools of phage obtained from all seven selections were amplified and tested for their reactivity to each of the selecting sera in ELISA. The results of this experiment showed a significant enrichment of phage recognised by the selector antibodies, as evidenced by the increase in reactivity with respect to the unselected library (Figure 2). In most cases, phage pools enriched by HCV sera reacted with more than one patient's serum. Peptides recognised by antibodies unrelated to HCV infection were also enriched from

the library. In fact, the pool of phage selected with the control serum has a higher reactivity with this serum than the unselected library (Figure 2). However, patients' sera drove selection toward HCV-related mimotopes as no reactivity to phage pools enriched by HCV sera was detected using sera from healthy individuals (Figure 2 and data not shown).

To gain insight into the frequency of reactivity of the selected mimotopes with different patients' sera, forty individual clones from two pools (4R and 2R, Table 1) were randomly chosen and tested for their reactivity in ELISA with a panel of twenty sera from HCV infected patients different from those used for the selection. An equivalent number of sera from non-infected healthy controls were used to assess the specificity for anti-HCV antibodies. Twenty-four clones turned out to be HCV-specific. Their distribution as a function of their frequency of reactivity with patients' sera is reported in Figure 3 (upper panel). Among them, phage reacting with more than one serum were identified; some of these were recognised by up to 55% of the tested sera.

To further improve the isolation of mimotopes reacting with many different anti-HVR1 antibodies, the enriched phage pools were subjected to a second round of affinity selection using patients' sera different from those used for the first round. In this way nine new pools were generated (Table 1) and

analysed by ELISA. As before, a general increase in reactivity with the selector antibodies was observed. In addition, all second round phage pools reacted more frequently than those selected in the first round with a panel of sera from HCV-infected patients different from those used for either selection, reflecting a higher recognition frequency of the isolated peptides. This was confirmed by comparing the reactivity with HCV sera of clones randomly chosen among those eluted after one round of affinity selection (Figure 3, upper panel) and those obtained by re-selecting them with a second different serum (Figure 3, lower panel). Not only the frequency, but also the distribution of reactivity appeared to be significantly different after the second selection step. While recognition of phage from the first selection appears to be rather scattered, clones isolated through two rounds of selection show a bell-shaped distribution of their frequency of reactivity with an average value of 60% (Figure 3, lower panel), indicating that the whole phage population had indeed acquired more of the desired binding properties. It was decided to omit additional selection cycles to avoid introduction of a bias toward biologically favoured phage during amplification.

A total of one hundred and seventy one clones reacting exclusively with HCV sera were identified by screening all

second-round pools. Their distribution as a function of the recognition frequency by HCV sera mirrored that of the subset displayed in Figure 3, lower panel, with the best clones reacting with 80% of the tested samples. More importantly, the profiles of reactivity of the selected mimotopes highlight another relevant feature. Despite their quantitative similar overall frequency of recognition by the HCV sera, different clones display a characteristic pattern of reactivity with the net result that few mimotopes can score for the presence of anti-HVR1 antibodies in all tested sera (Figure 4A).

Next, it was verified whether the observed high frequency of recognition by HCV sera was limited to the tested patients' population or whether it reflected an intrinsic property of the selected mimotopes. For this purpose another set of sera from infected patients was assayed by ELISA revealing that both the frequency of reactivity of each individual phage and the total coverage of the sera remained unaltered (Figure 4B).

HCV infected individuals who have resolved the infection most likely came in contact with a lower number of viral variants and presumably developed a narrower spectrum of variant-specific anti-HVR1 antibodies than chronically infected patients. This is supported by the finding that sera from the former population react with synthetic peptides reproducing the HVR1 of natural isolates much more rarely than those of

chronically infected viremic patients (Scarselli et al., 1995). Therefore, non viremic sera could constitute a better and more stringent test for assaying the cross-reactivity of HVR1 mimotopes with different anti-HVR1 antibodies. Some of the selected mimotopes were thus tested against forty-one samples from HCV seropositive individuals who were repeatedly found negative for the presence of viral RNA in the blood. Again, the mimotopes reacted with many of these sera albeit at a lower frequency than that observed with sera from viremic patients (compare Figures 4(A), 4(B) and 4(C)). These data provide an indication of the ability of the selected mimotopes to cross-react with a large number of different anti-HVR1 antibodies.

EXAMPLE 3 - Determination of a relationship between the sequence of the selected HVR1 mimotopes and their frequency of reactivity with HCV sera.

The inventors wished to verify whether the amino acid sequence of the selected clones correlates with their frequency of reactivity. No obvious pattern arises from a visual comparison of the sequences so it was decided to analyse separately the sequence patterns of the least and most frequently reacting clones.

Defined as "weak" were the 24 clones that only reacted with less than 3 sera and defined as "strong" were the 27